ELECTRON MICROSCOPIC STUDY OF THE ADRENAL CORTEX : ESPECIALLY THE INFLUENCE OF ESSENTIAL FATTY ACID DEFICIENCY ON ADRENOCORTICAL STRUCTURE

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by

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I. INTRODUCTION

In connection with the recent studies in our laboratory on the process of fat metabolism in vivo and its nutritional effects, we are now faced with the problem of clarifying the physiological significance of essential fatty acids (abbreviated as EFA).[14~18, 25, 35, 58]

Recent studies by our colleagues MATSUDA and NAGASE have, for the first time, shown that EFA deficiency may be one of the most important factors causing a decrease in adrenocortical function. In his study on the changes of liver glycogen content during fasting, MATSUDA has revealed that EFA-deficient animals show an earlier exhaustion of liver glycogen content and succumb to starvation sooner than normal animals, and has suggested that the main cause of these phenomena is a decrease in adrenocortical function due to EFA deficiency.[36, 37] NAGASE's experimental study on acute postoperative pulmonary edema revealed that EFA deficiency causes an abnormal increase in capillary permeability predisposing to acute postoperative pulmonary edema in organisms undergoing operative insult not only because of structural changes in the capillary wall but also because of decreased adrenocortical function.

Our colleagues MAKI, JINDO, NAKASHIO, KUMANO et al. have demonstrated that the adrenals contain more EFA than do any other organs and show not only a very typical pattern of deficiency of adrenocortical EFA but also specific changes in adrenocortical EFA content induced by stresses such as ACTH injection or starvation.[23, 27, 35, 39] In view of these experimental results, it has been postulated that EFA have a very intimate relationship with the biosynthesis of glucocorticoids in the adrenal gland. Actually, in his study on the changes of glucocorticoids in the urine and blood of EFA-deficient animals under various stresses, our colleague TAMAKI has shown that the adrenocortical function of EFA-deficient animals is much poorer than that of the controls.[57] Furthermore, MATSUDA's light microscopic and histochemical investigations on the adrenal cortex have shown that the adrenal cortex of EFA-deficient animals undergoes early exhaustive changes in the starvation test.[36] These findings indicate that there is an intimate relationship between EFA and the adrenocortical function, especially the biosynthesis of glucocorticoids.

In the present study, therefore, electron microscopic investigations were performed in order to clarify the structural changes in the adrenal cortex in EFA-deficient animals under various conditions and correlate these with the results of the biochemical and histochemical investigations conducted in our laboratory. This study is limited to the fat

granules, mitochondria, endoplasmic reticulum and vacuole formation in the cells of the zona fasciculata.

Ⅱ. EXPERIMENTAL ANIMALS AND METHODS

A. Experimental Animals

Male albino rats of the Wistar strain supplied by the Animal Center of Kyoto University were fed with the standard diet (rat chow, produced by ORIENTAL Yeast Ind. Co. Ltd., Japan) until their weights reached 50 to 60 grams and were then fed with one of the following diets for about 3 months at a room temperature of 20°C.

1. Fat-free diet
2. Fat diet
3. Standard diet

The weight composition of each diet is as follows. Fat-free diet : casein 20 %, starch 76%, mixed salts 4 % and vitamin mixture 0.6g/100g of food. Fat diet : casein 20%, starch 61 %, sesame oil 15 %, mixed salts 4 % and vitamin mixture 0.6g/100g of food.

According to our colleague JINDO, each gram of the casein used in the study contained 1.4 mg of total lipids and 0.2 mg trienoic acid and no other unsaturated fatty acids.[23] The lipid content of the starch used was less than 0.01 %. Therefore, a rat eating 10g of the fat-free diet per day ingests less than 0.4mg of EFA per day. It is evident from NAGASE's and JINDO's studies that EFA deficiency is induced by feeding animals with this fat-free diet for about 3 months.[23, 37] The purified sesame oil used in this study contains 40.4 % linoleic acid and is free of peroxide. Therefore, a rat eating 10g of the fat diet daily takes in about 600mg of linoleic acid per day. The daily intake of EFA of a rat eating 10g of the standard diet per day is less than 60mg, as the content of EFA in the standard diet is 0.59 %.

B. Experimental Methods

Having stimulated the adrenal cortex by treatment with such stresses as formalin injection, starvation, ACTH injection and hypophysectomy, these animals were sacrificed by a head-blow. Both adrenals were removed immediately, and several tissue blocks were placed in 1 % osmic acid solution which was maintained at pH 7.2 with a veronal buffer solution. After fixing for 21/2 hours in a refrigerator, the blocks were, without washing, serially dehydrated in alcohol, embedded in a mixed solution of n-butyl- and methyl-methacrilate (6 : 4), and kept in a refrigerator for 24 hours. The solution was then tubed into No. 0 capsules, embedded, and heated at 55°C in an incubator for polymerization. Thin sections 15 to 30 mμ thick were made with a NIPPON ultramicrotome and were observed with an AKASHI Tronscope TRS-50 E-type. Photographs were taken at 1000 to 6000 magnifications.

Ⅲ. OBSERVATIONS

A. Ultrafine Structure of Adrenal Cortex in Resting State

Since the adrenal cortex consists of three zones, the intracytoplasmic features show a certain amount of individual variation electron microscopically even under resting conditions. In the present investigation, therefore, many animals were used for each

experiment, and the attempt was made to determine only the common findings.

The general appearance of the fasciculata cells of rats on the standard diet in the resting state is presented in plate 1. In the intracytoplasmic organellae commonly present in the zona fasciculata there are fat granules, vacuoles, microbodies, Golgi apparati etc., besides nucles, mitochondria and endoplasmic reticulum.[1, 6, 9, 22, 42, 45, 49] The nucleus is usually situated in the center of the cell outlined by a dense double membrane, the inner component of which is filled with fairly dense granular material, presumably chromatin.[56, 60] The nuclei are nearly oval in shape and in some of them nucleoli can be seen clearly. Mitochondria have a very characteristic inner structure in the adrenal cortex to be described more precisely later and are seen as round particles 0.5 to 1.0μ in size, relatively many in number and diffusely dispersed in the cytoplasm. The endoplasmic reticulum in the adrenal cortex is all smooth-surfaced.[41, 51]

The intracytoplasmic organellae in the fasciculata cells show considerable differences between the fat and fat-free diet groups in the resting state, chiefly in the distribution of fat granules and in the inner structure of the mitochondria. In the fat-free diet group the mitochondria vary in shape and have an irregular inner structure, and the fat granules are fewer but not totally absent (Plates 2 and 3).

B. Ultrafine Structure of Adrenal Cortex under Various Conditions

1.) Formalin Injection Test

1.0ml of 4 % formalin solution was injected intraperitoneally, and the animals were sacrificed after 2, 6, 12 and 18 hours.

a.) 2 hours later

In the fat diet group there was a relative increase in the number of mitochondria, developed smooth surfaced endoplasmic reticulum and many fat granules 0.2 to 0.7μ in size, while in the fat-free diet group none of these changes were seen, but there were osmiophilic granules of low density 0.5 to 1.5μ in diameter, quite different from fat granules. These are considered to represent a stage in the degeneration of mitochondria predisposing to vacuolization (Plates 4 and 5).

b.) 6 hours later

The differences between the two groups were further intensified at this stage. In the fat-free diet group a few vacuoles 1.0 to 2.5μ in diameter were seen in the cytoplasm of each cell but very few fine fat granules. In the fat diet group there was no appreciable difference from the appearance at 2 hours (Plates 6 and 7).

c.) 12 hours later

In the fat diet group the cells resembled those examined at 6 hours except for a further increase in the number of fat granules. In the fat-free diet group there was much more mitochondrial vacuolization and no fat granules (Plates 8 and 9).

d.) 18 hours later

In the fat diet group, the above-mentioned successive changes of intracytoplasmic organellae showed a tendency to return to the normal pretreatment state, while in the fat-free diet group there was a continuation of exhaustive changes in the intracytoplasmic organellae.

2.) Starvation Test

The animals subjected to this stress were separated from each other and given only adequate water at the same room temperature. They were killed on the 2nd, 5th, 7th and 12th days of starvation.

a.) On the 2nd day of starvation

In the fat-free diet group there was vacuole-formation, a relative decrease in number and size of mitochondria and a notable decrease in the number of fat granules. In the fat diet group there was a relative increase in number of mitochondria and a slight decrease of fat granules, but no vacuolization (Plates 10 and 11).

b.) On the 5th day of starvation

The differences between the two groups were more definite. In the fat-free diet group there was a decrease in the number of mitochondria as well as some structural changes, definite vacuolization and a few fat granules. In the fat diet group also there was a slight decrease in the number of mitochondria and some structural changes, but not to the extent of mitochondrial vacuolization; fat granules 0.5 to 1.5μ in diameter were still clearly observed (Plates 12 and 13).

c.) On the 7th day of starvation

The differences between the two groups were further intensified. In the fat-free diet group there was a still greater decrease in number of mitochondria, disintegration of their inner structure, vacuolization and a depletion of endoplasmic reticulum. The exhaustive changes due to starvation were less marked in the fat diet group. A few fat granules were still present in the fat diet group but none in the fat-free diet group.

d.) On the 12th day of starvation

When starvation had continued to this stage, the differences between the two groups were much more pronounced. The fat-free diet group showed marked cytolysis, a large number of vacuoles and depletion of the cytoplasmic organellae, whereas, the fat diet group still showed well balanced changes similar to those seen on the 7th day of starvation (Plates 14 and 15).

3. ACTH Injection Test

The effects of ACTH injection on adrenocortical structure both electron and light microscopically have already been reported and discussed by many authors, namely SELYE, DEAN, MILLER, OOI, YOSHIMURA, SAKAMOTO et al.[8, 10, 46, 51, 52] In the present investigation, after injection of ACTH in doses of 5 to 10mg intraperitoneally, the adrenal glands of these animals were removed periodically at hourly intervals and studied electron microscopically.

a.) 2 hours after ACTH injection

The fat diet group showed a great increase in the number of mitochondria development of endoplasmic reticulum and a slight increase in the number of fine fat granules. In the fat-free diet group there were large fat granules 2.5μ in diameter, which are never seen in the resting state, but there was no increase in number of mitochondria or development of endoplasmic reticulum. In short, the greatest difference between the two groups was in the number of mitochondria (Plates 16 and 17).

b.) 6 hours after ACTH injection

The differences between the two groups mentioned above were still greater; i. e.,

the number of mitochondria, changes in fat granules and formation of vacuoles. In the fat diet group, in addition to the findings noted 2 hours after ACTH injection, there were many fat granules 0.3 to 1.2μ in size which were nearly equal to those observed in the rest-ing state. In the fat-free diet group there were changes in the inner structure of the mitochondria, marked vacuolization and no fat granules (Plates 18 and 19).

c.) 12 hours after ACTH injection

In the fat diet group the cells resembled those seen 6 hours after ACTH injection but in the fat-free diet group the abnormal findings described above had progressed still further (Plates 20 and 21).

d.) 24 hours after ACTH injection

The fat diet group tended to return to the resting state, while the fat-free diet group still continued to show the above-mentioned cellular exhaustive changes : abnormal mitochondrial structure, depletion of endoplasmic reticulum, vacuolization, etc. (Plates 22 and 23).

e.) 48 hours after ACTH injection

The fat diet group had returned almost completely to the resting state, while the fat-free diet group still showed progression of exhaustive changes (Plates 24 and 25).

Ⅳ. SUMMARY AND DISCUSSION

There have been many studies of the relationship between function and structure of the adrenal cortex.[2, 24, 30, 31, 32, 62] SELYE reported that the morphological changes of the adrenal cortex following various stresses were atrophy, hypertrophy, bleeding, hyperplasia, cytolysis, necrosis, fatty degeneration, storage or discharge of various granules etc., in accordance with the kinds of stress or the conditions of the organism, and that the development of fine fat granules was especially related to adrenocortical hormone production.[52] Furthermore, DEAN and DALTON stated that biosynthesis of steroid hormone, an original function of the adrenal cortex, was closely related to the fine fat granules in the cytoplasm.[10]

It has also been well known that the adrenal cortex contains a large amount of lipids, which consist mainly of cholesterol, especially esterified cholesterol, which is considered to be a precursor of adrenocortical hormones.[4, 19]

Recently, our colleagues MAKI and JINDO by measuring the EFA content of various organs in the body with paperchromatography and alkaline isomerization respectively, have demonstrated that the adrenal cortex contains the largest amount of EFA per volume of tissue in the body.[23, 35]

Moreover, it has been a general concept that cholesterol must be esterified with EFA in order to be introduced into normal metabolism and that if it is bound with other unsaturated fatty acids, it becomes inactive and is deposited within the tissues. Therefore, it is evident that cholesterol must be esterified with EFA in order to be converted to steroid hor mone. As mentioned above, it has been clarified by our colleagues MATSUDA and NAGASE that adrenocortical function is certainly decreased in EFA-deficient organisms.[36, 37] MATSUDA studied the changes of liver glycogen content and the histological changes in the adrenal cortex during fasting and has revealed that EFA-deficient animals subjected to starvation for a period, show early consumption of liver glycogen, fail to maintain

homeostasis and show from an early stage highly exhaustive changes in the adrenal cortex, such as appearance of vacuoles, cellular disintegration etc. Moreover, NAGASE has demonstrated that EFA deficiency, on the one hand induces structural changes in the capillary walls, and on the other hand causes a decrease of adrenocortical function. These easily lead to acute postoperative pulmonary edema following overhydration or various stresses such as operative insult. Furthermore, our colleague TAMAKI, in his study on adrenocortical function by measuring the urinary formaldehydogenic corticocids and the plasma fluorometric corticoids in rats on a fat-free diet, showed that these levels are very low not only under various stresses but also at rest in comparison with those in rats on a fat diet, and that the adrenocortical function of EFA-deficient rats is greatly decreased.

It is apparent from these experimental results that EFA have a most important role in the biosynthesis of adrenocortical hormone. Since the adrenocortical function is greatly decreased in EFA-deficient organisms, their adrenal cortices can not respond adequately to the hormonal demands on them.

This study was designed, therefore, to correlate these biochemical and histochemical results with the electron microscopic appearance of the adrenal cortex, especially the zona fasciculata, of rats fed with various diets under various conditions.

There have been many studies of functional localisation in the adrenal cortex,[2] and recent advances in histochemical techniques have confirmed that the zona glomerulosa is primarily responsible for secretion of mineralocorticoids, the zona fasciculata for secretion of glucocorticoids and the zona reticularis is chiefly the source of androgen production.[12] Recent studies in our laboratory have been concerned with the biosynthesis and secretory function of glucocorticoids in the adrenal cortex; so the present investigation deals only with the zona fasciculata. The relationship between EFA and the biosynthesis of mineralocorticoids in the zona glomerulosa will be investigated in a subsquent study.

In this study the changes in intracytoplasmic organellae were chiefly in the mitochondria. Under light microscopy mitochondria were seen as ubiquitous intracellular bodies of filamentous, rodlike or granular form with cetain well-defined staining characteristics. More recent biochemical studies of mitochondrial fractions separated by differential centrifugation from tissue homogenate have demonstrated that mitochondria possess a complex chemical composition and remarkable enzymatic activity in vitro.[7, 11, 26, 44] Elecrton microscopic investigation has further clarified the fact that mitochondria in all animal cells are bounded by a membrane and have a system of parallel, regularly spaced ridges that protrude from the inside surface of the membrane towards the interior.[13, 44, 53, 59, 61] PALADE called this membrane-like structure "crista mitochondrialis".[44] However, the mitochondria in the adrenocortical fasciculata cells characteristically show a rather alveolar structure without cristae, as is seen in each plate. It is very noteworthy that mitochondria with this special structure, are observed, in addition to the adrenal cortex, only in the cytoplasm of the so-called steroid hormone secreting organs, such as the corpus luteum, granulosa of Graafian follicles, theca interna etc.[5, 51] Mitochondria with this inner structure specific for adrenocortical fasciculata cells, in agreement with the experimental results clarified by our colleague TAMAKI, have a very irregular inner structure and vary in size even in the resting state in the fat-free diet group, i. e., in EFA-deficient rats. In these EFA-

deficient animals there is a marked decrease in the number of mitochondria along with changes in their inner structure under such stresses as formalin injection, in the ACTH injection; i. e., the alveolar structure of mitochondria in the resting state are transformed state into a large number of huge vacuoles after becoming irregular in outline, disintegrating, disappearing and finally undergoing vacuolization. This type of change seems to represent an exhaustion phenomenon of the adrenal cortex, since it resembles that seen in hypophysectomized rats, as illustrated in plates 26 and 27.

Hitherto, cytoplasmic vacuolization has been considered to be a "degenerative process" such as cellular exhaustion or disintegration, both light and electron microscopically. However, SAKAMOTO reported that various vacuoles in the adrenal cortex were observed not only in the degenerative process but also in relation to adrenocortical hormone secretion, and she divided them into the following three types: true vacuoles, functional vacuoles and degenerative vacuoles.[51] In the present investigation physiological vacuoles in intact animals in the resting state were definite, but findings supporting the concept that these vacuoles occur in relation to adrenocortical hormone production could not be obtained. Hence, it has been considered that the vacuole formation seen in the zona fasciculata of EFA-deficient animals in various states should be regarded as a kind of exhaustive change due to mitochondrial degeneration. Actually, in agreement with the results reported by our colleague TAMAKI, the rats fed with the fat diet in this study maintainedtheir adrenocortical function at a normal level without any increase in the number of physiological vacuoles or any vacuolization due to mitochondrial degeneration. When glucocorticoids secretion is extremely advanced, mitochondria rather increase in number simultaneously with the development of endoplasmic reticulum, and the inner structure of the mitochondria returns sooner to the pretreatment reactive resting state without showing any exhaustive changes. But the rats on the fat diet subjected to starvation stress, also showed a temporary increase in number of mitochondria during prolonged fasting and thereafter a decrease. At the same time, changes in the mitochondrial inner structure were noted, but these were not so marked as the mitochondrial vacuolization seen in the fat-free diet group. The fat fed animals retained a comparatively normal balance of intracytoplasmic organellae.

In process of mitochondrial vacuolization, as illustrated in plate 23, some less dense osmiophilic granular substance was seen as disintegration of the inner structure progressed. Varied opinions have been proposed in regard to this pigmentation observed in mitochondria. LOW and PALADE interpreted these structural changes as one step of the functional process of mitochondria.[33, 44] ROBERTIS, SJOSTRAND et al., on the other hand, considered this substance to represent mitochondrial degeneration, in agreement with our opinion.[47, 53] Actually, this phenomenon was often observed in the mitochondria of the adrenocortical fasciculata cells of rats on the fat-free diet, in which adrenocortical function is decreased, while in those on the fat diet such findings were rarely observed.

Consequently, as pointed out by MILLER, it is postulated that in rats on the fat diet, in which adrenocortical function remains normal, mitochondria in the adrenocortical fasciculata cells increase in number in parallel with their cellular activity and play a most important role in glucocorticoid production.[46] On the other hand, in rats on the fat-free diet, in which adrenocortical function is extremely decreased due to EFA deficiency,

mitochondria are rather decreased in number, often irregular in structure, and finally become vacuolizated and are exhausted. The changes of smooth-surfaced endoplasmic reticulum also parallel those of the mitochondria in rats on the fat diet, and when mitochondria increase in number, preceding increased cellular activity, the endoplasmic reticulum also shows development. However, in the cytoplasm of rats on the fat-free diet, when mitochondria decrease in number and disintegration of their inner structures occurs under various conditions, the enodplasmic reticulum is also poorly developed and is often depleted. Furthermore, fine fat granules recognized in the cytoplasm of rats on the fat diet are abundant in number in comparison with those of rats on the fat-free diet even at rest. So, once various stresses such as ACTH injection or formalin injection are applied, these mitochondria retain their structure fairly well throughout the entire period, and even if they are somewhat decreased in number temporarily, as time goes on they return to their former state and then go on to increase further. In short, in rats on the fat diet, which contains a large amount of EFA, when the various stresses mentioned above are imposed upon the organism, these EFA are mobilized immediately into the adrenal glands and transformed into cholesterolester with high metabolic activity. It has been considered that adrenocortical function recovers from the damage induced by such a mechanism. In starvation stress, rats on the fat diet also show a decrease of fine fat granules but the balance of intracytoplasmic organellae is well preserved even until death. On the other hand, in rats on the fat-free diet these fine fat granules are greatly decreased not only under starvation stress, but also after ACTH or formalin injection, and often they disappear completely or show remarkable exhaustive changes and do not increase again throughout the experiment. Nevertheless, in rats on the fat-free diet some gross fat granules are sometimes seen; these fat granules are considered not to be the fine fat granules observed in the cytoplasm under normal conditions but to be fat granules with no metabolic activity. That is, the gross fat granules seen in the cytoplasm by light microscope has been regarded as a kind of exhaustive change, and electron microscopically they may also be regarded as a kind of exhaustive change.

V. CONCLUSIONS

(1) The adrenocortical fasciculata cells of EFA-deficient animals at rest show variation in size of mitochondria and a tend to irregularity of their inner structure and, of course, fewer fine fat granules than do those of normal animals.

(2) The adrenocortical fasciculata cells of EFA-deficient animals at rest show early exhaustive changes of the intracytoplasmic organellae and a delayed return to the pretreatment reactive state.

(3) The main exhaustive changes are decrease in number of mitochondria and changes in their inner structure, vacuolization, depletion of smooth-surfaced endoplasmic reticulum, disappearance of fine fat granules, development of gross fat granules, etc..

(4) on the other hand, animals fed with a fat diet so that adrenocortical function is kept in a healthy condition, show a great increase in the number of mitochondria and development of endoplasmic reticulum and fine fat granules in parallel with the hyperactivity of the pituitary adrenocortical system, and, as time goes on, without showing either

total disappearance of these fine fat granules or the development of gross fat granules, they return sooner to their pretreatment reactive state.

(5) Therefore, in order to keep the adrenocortical function normal, EFA should be given in sufficient quantities. It might be said that the adrenocortical function in the living organism depends largely upon the amount of EFA present in the adrenal cortex.

(6) The inner structure of mitochondria in the adrenocortical fasciculata cells, shows electron microscopically the same structure that is seen in the organs concerned with the synthesis of steroid hormones, which is very different from that seen in other organs. Consequently, it is considered that the mitochondria in the adrenocortical fasciculata cells play an important role in glucocorticoid production.

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ELECTRON MICROGRAPHS OF ADRENAL CORTEX

SYMBOLS

CM : cell membrane

DC : dark cell

E : erythrocyte

FG : fat granule

M : mitochondria

NC : nucleus

NCL : nucleolus

SER : smooth surfaced endoplasmic reticulum

V : vacuole

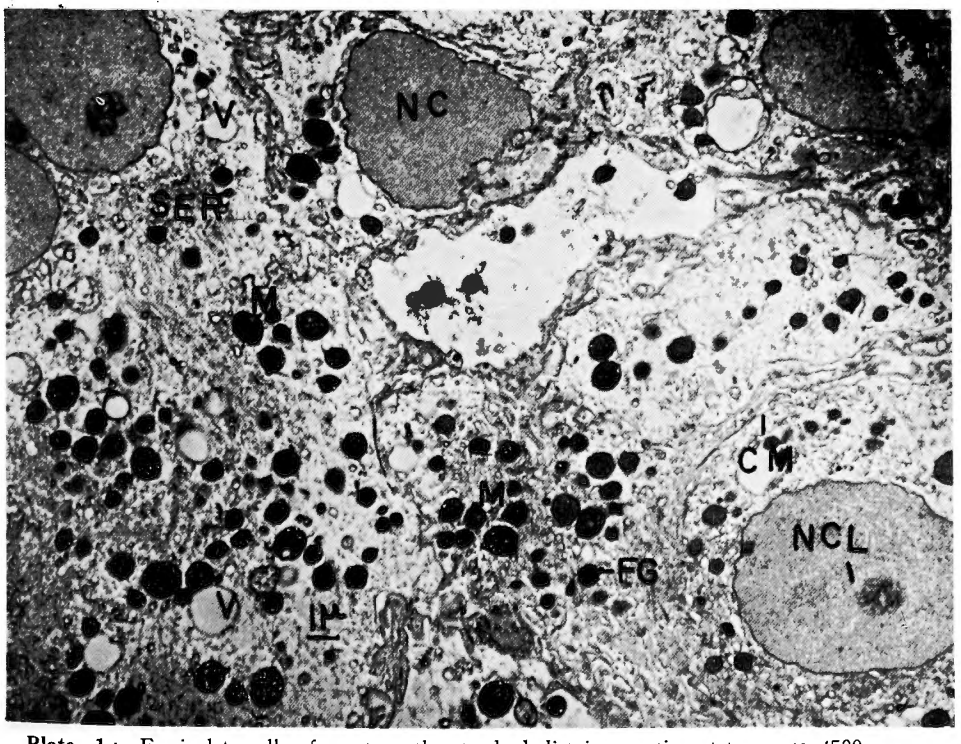

Plate 1 : Fasciculata cells of a rat on the standard diet in a resting state. × 4500

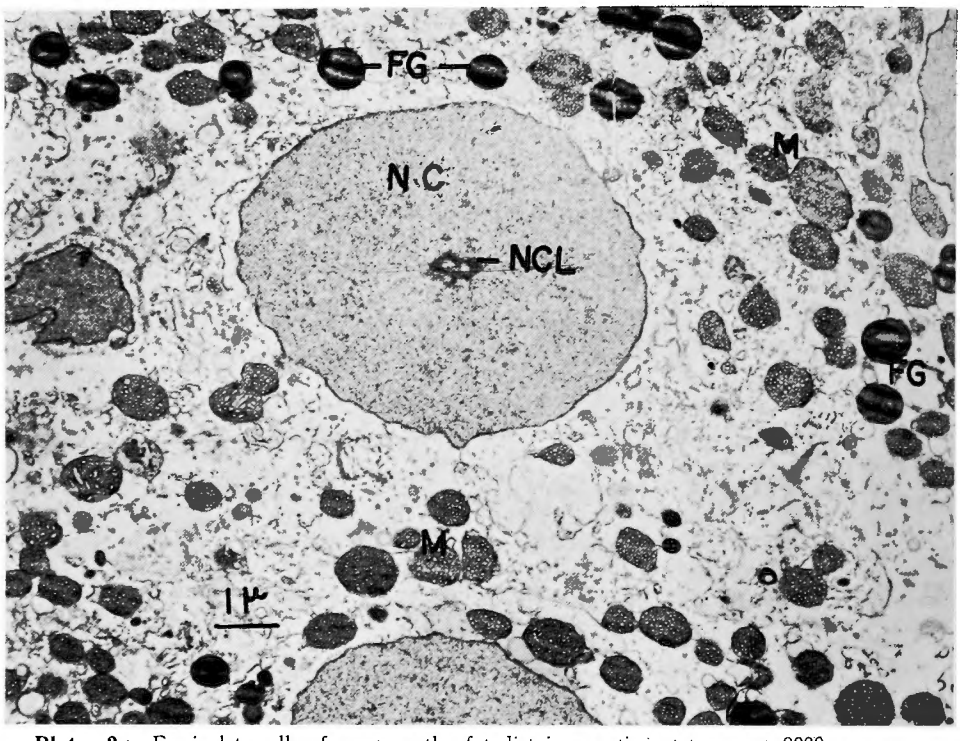

Plate 2 : Fasciculata cells of a rat on the fat diet in a resting state. × 9000

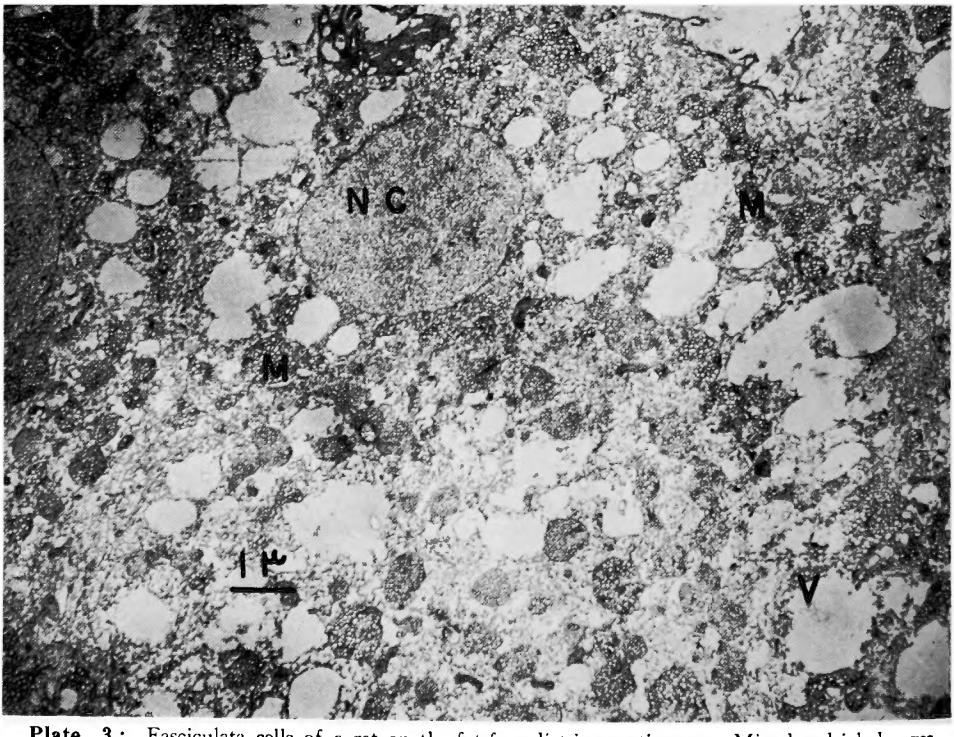

Plate 3 : Fasciculata cells of a rat on the fat-free diet in a resting state. Mitochondrial changes in both shape and structure are noted and fine fat granules are rarely seen. ×9000

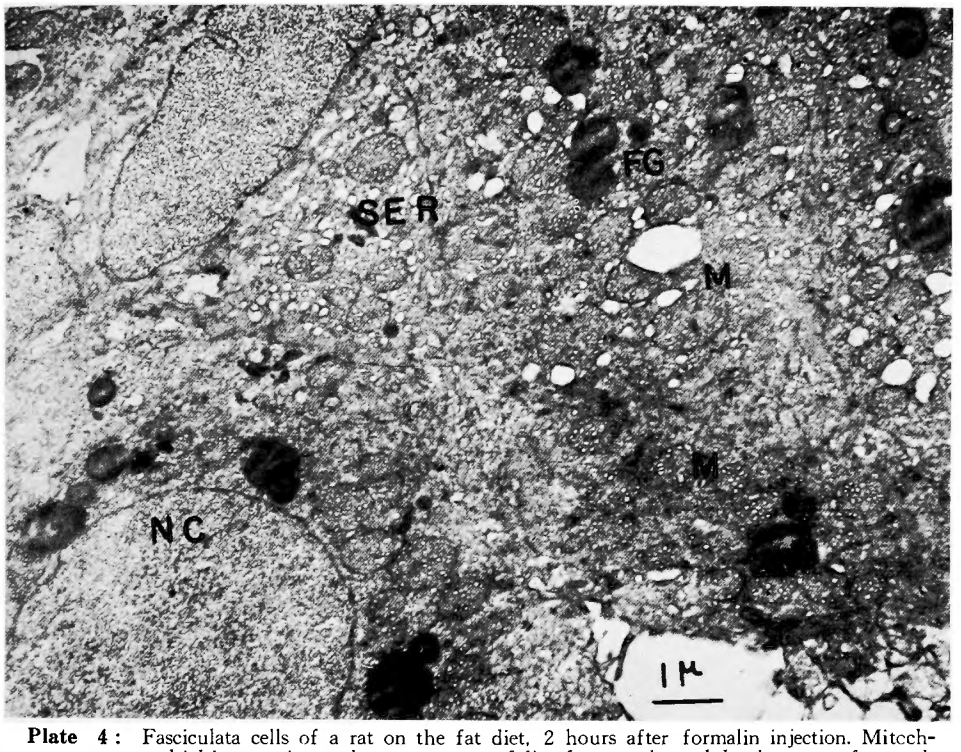

Plate 4 : Fasciculata cells of a rat on the fat diet, 2 hours after formalin injection. Mitochondrial increase in number, occurrence of fine fat granules and development of smooth surfaced endoplasmic reticulum are noted. × 9000

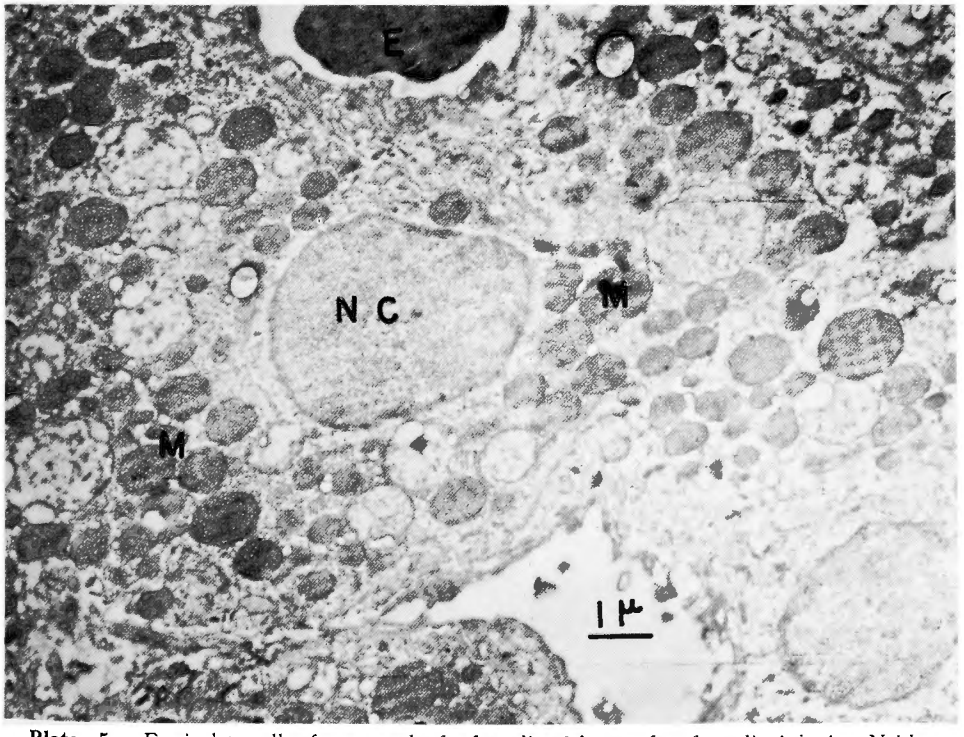

Plate 5 : Fasciculata cells of a rat on the fat-free diet, 2 hours after formalin injection. Neither increase in number of mitochondria nor occurrence of fine fat granules is seen. ×9000

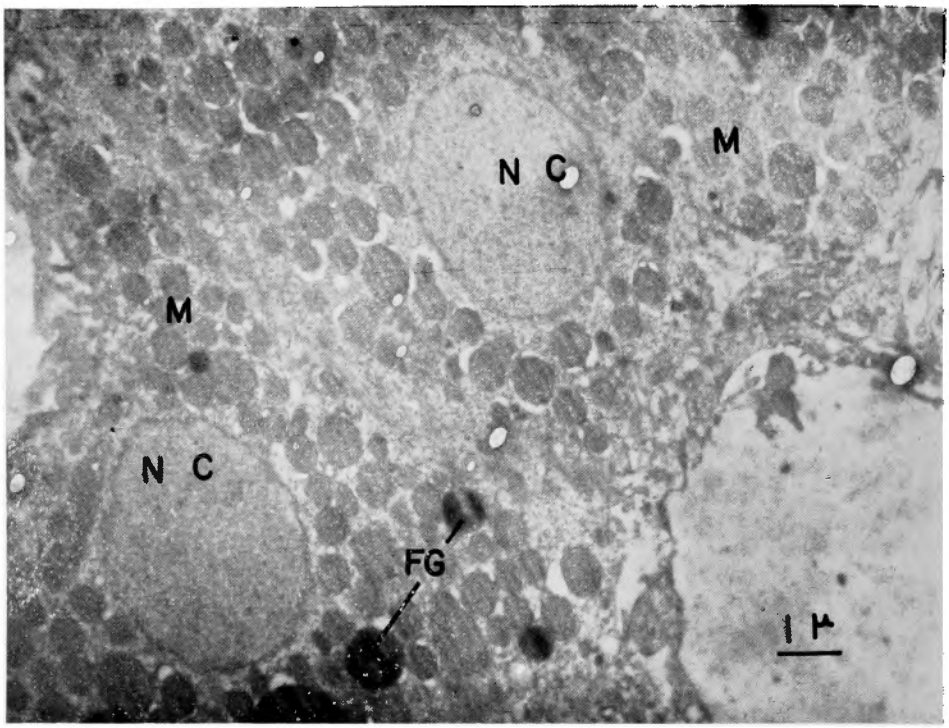

Plate 6 : Fasciculata cells of a rat on the fat diet, 6 hours after formalin injection. × 9000

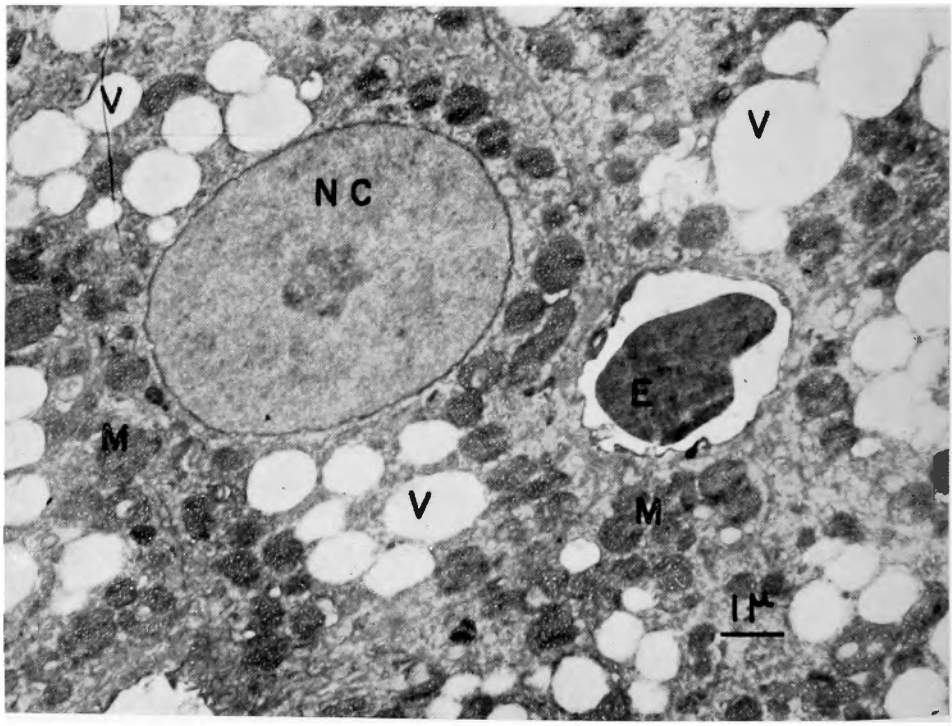

Plate 7 - Fasciculata cells of a rat on the fat-free diet, 6 hours after formalin injection. In some parts of the cytoplasm, a few vacuoles 1.0 to 2.0 μ in diameter are seen. ×9000

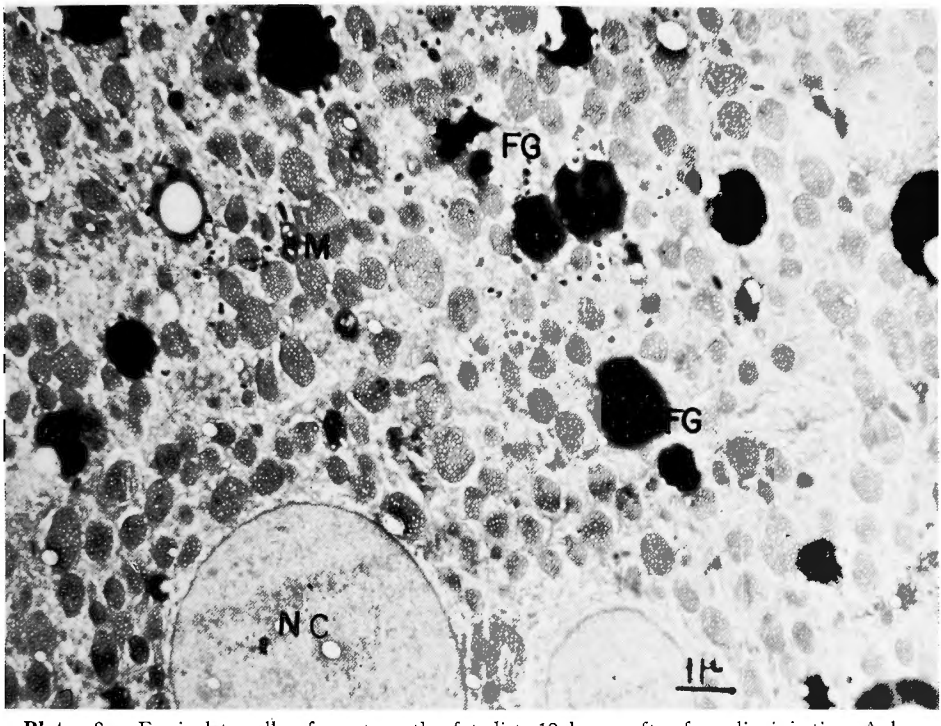

Plate 8 : Fasciculata cells of a rat on the fat diet, 12 hours after formalin injection. A large number of electron-dense fine fat granules are noted. × 9000

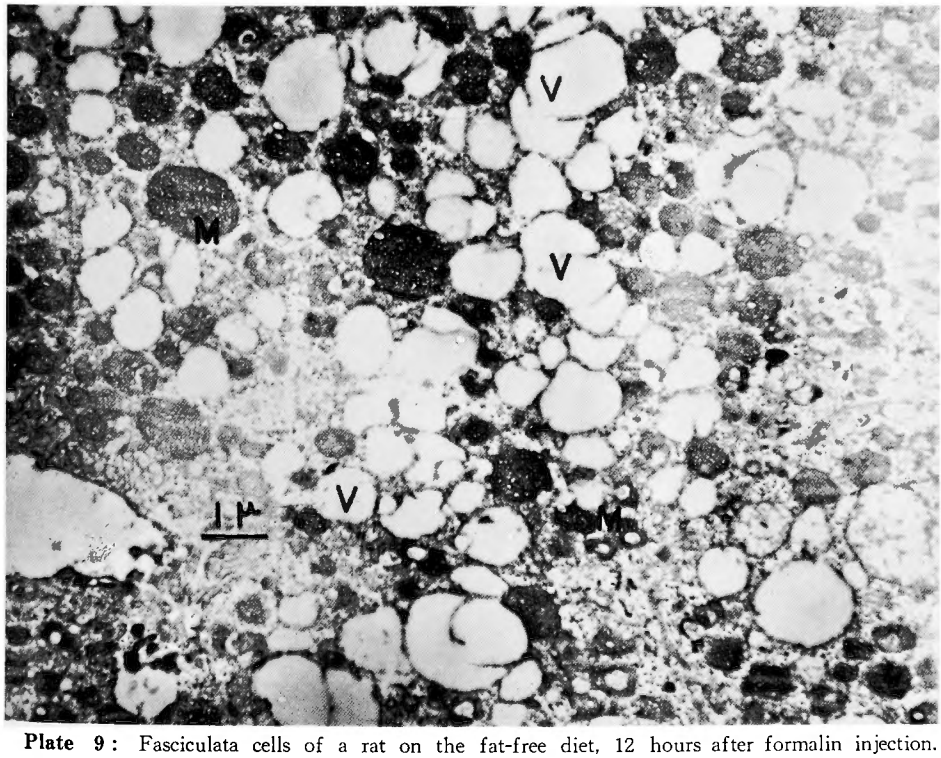

Plate 9 : Fasciculata cells of a rat on the fat-free diet, 12 hours after formalin injection. Mitochondrial vacuolization and structural changes are noted. × 9000

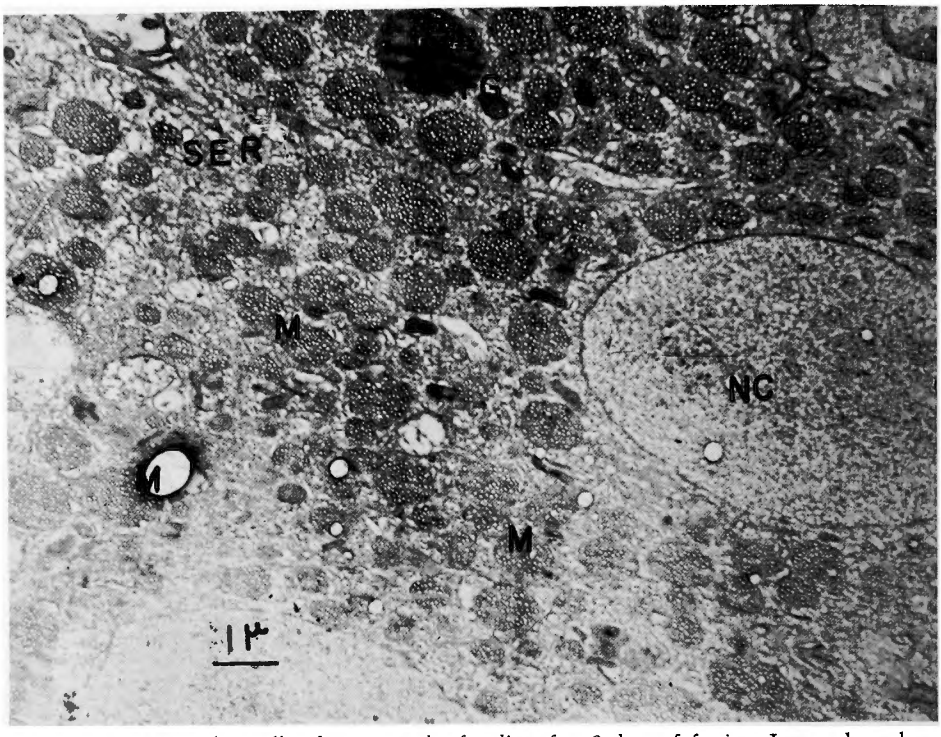

Plate 10 : Fasciculata cells of a rat on the fat diet after 2 days of fasting. Increased numbers of mitochondria are noted. × 9000

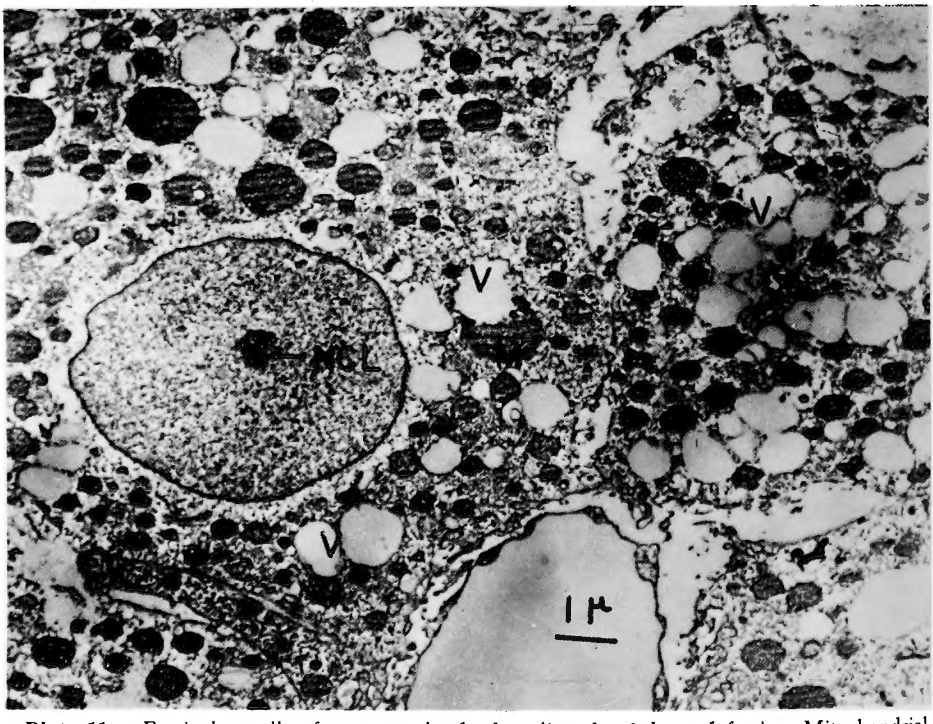

Plate 11 : Fasciculata cells of a rat on the fat-free diet after 2 days of fasting. Mitochondrial vacuolizations are already developing. × 9000

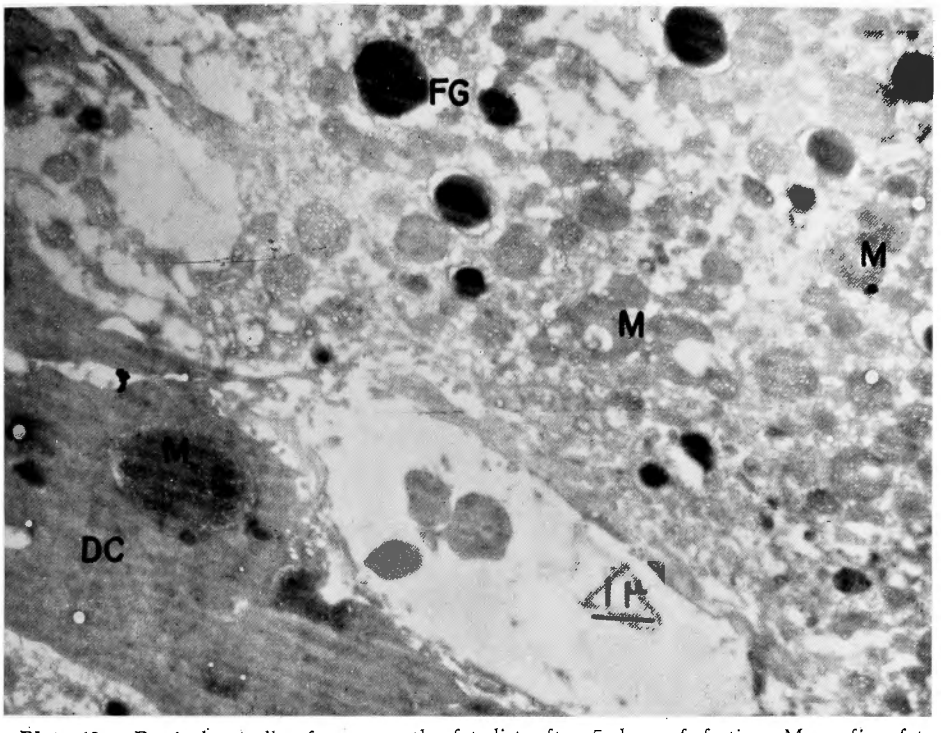

Plate 12 : Fasciculata cells of a rat on the fat diet after 5 days of fasting. Many fine fat granules are seen in the cytoplasm. × 9000

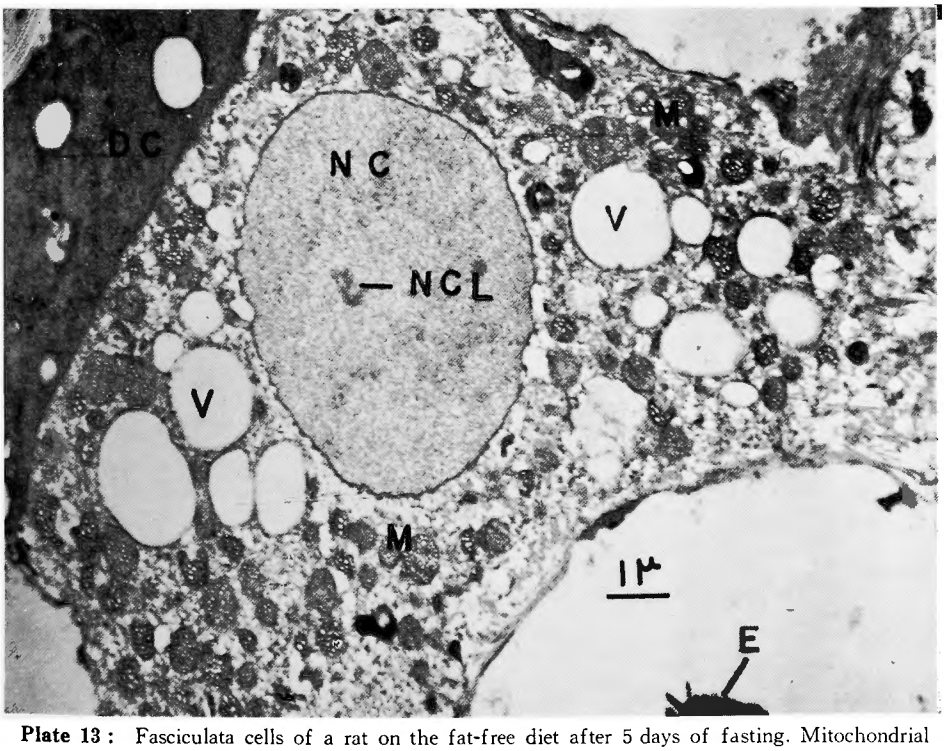

Plate 13 : Fasciculata cells of a rat on the fat-free diet after 5 days of fasting. Mitochondrial vacuolization are larger than those seen after 2 days of fasting. × 9000

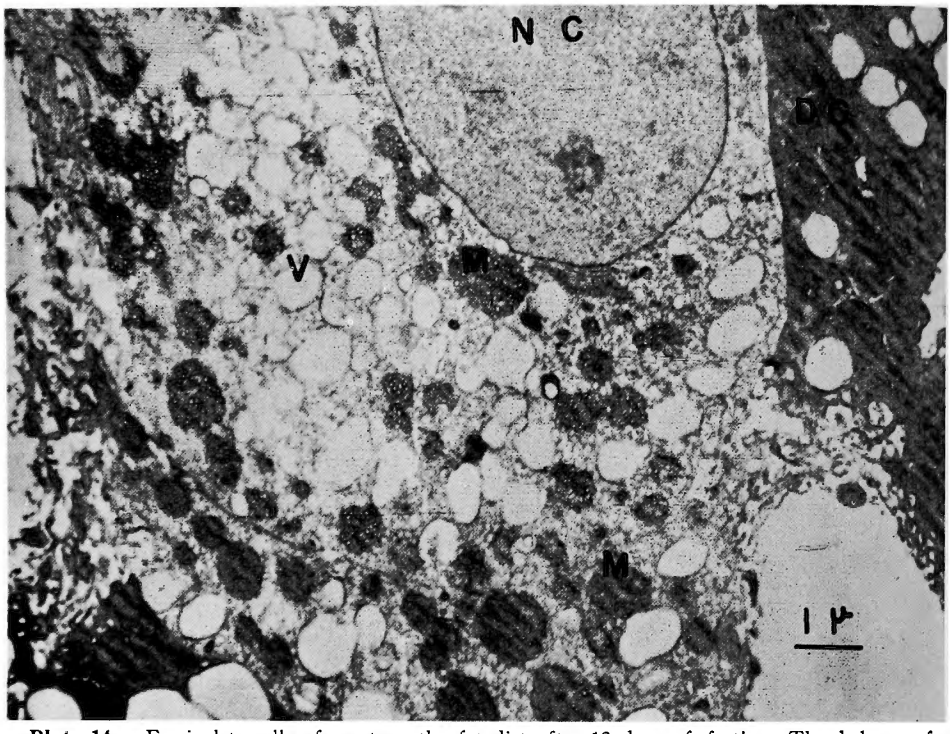

Plate 14 : Fasciculata cells of a rat on the fat diet after 12 days of fasting. The balance of intracytoplasmic organellae is well preserved as compared with that of a rat on the fat-free diet illustrated in plate 15. × 9000

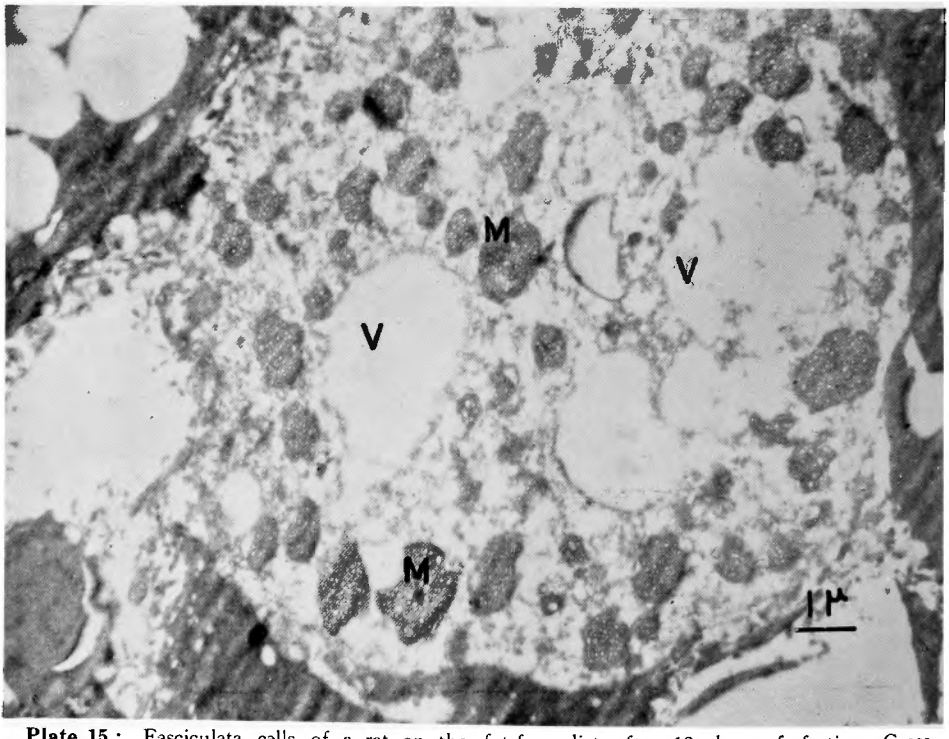

Plate 15 : Fasciculata cells of a rat on the fat-free diet after 12 days of fasting. Gross vacuoles and mitochondrial changes predisposing to cytolysis are noted. × 9000

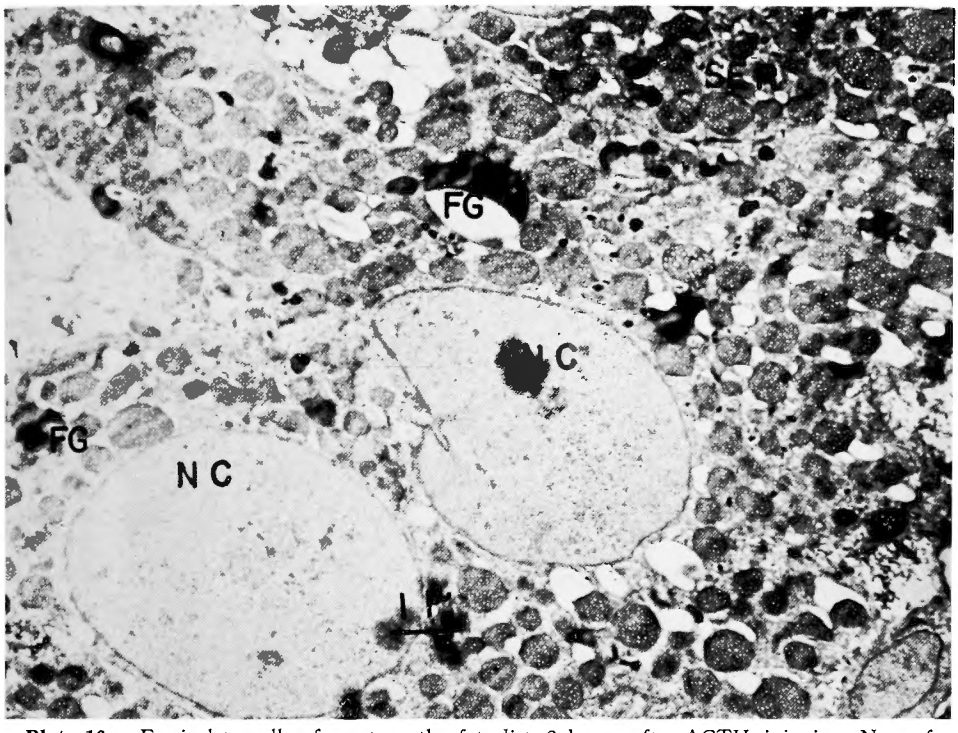

Plate 16 : Fasciculata cells of a rat on the fat diet, 2 hours after ACTH injection. Note of increase in number of mitochondria and occurrence of fine fat granules. × 9000

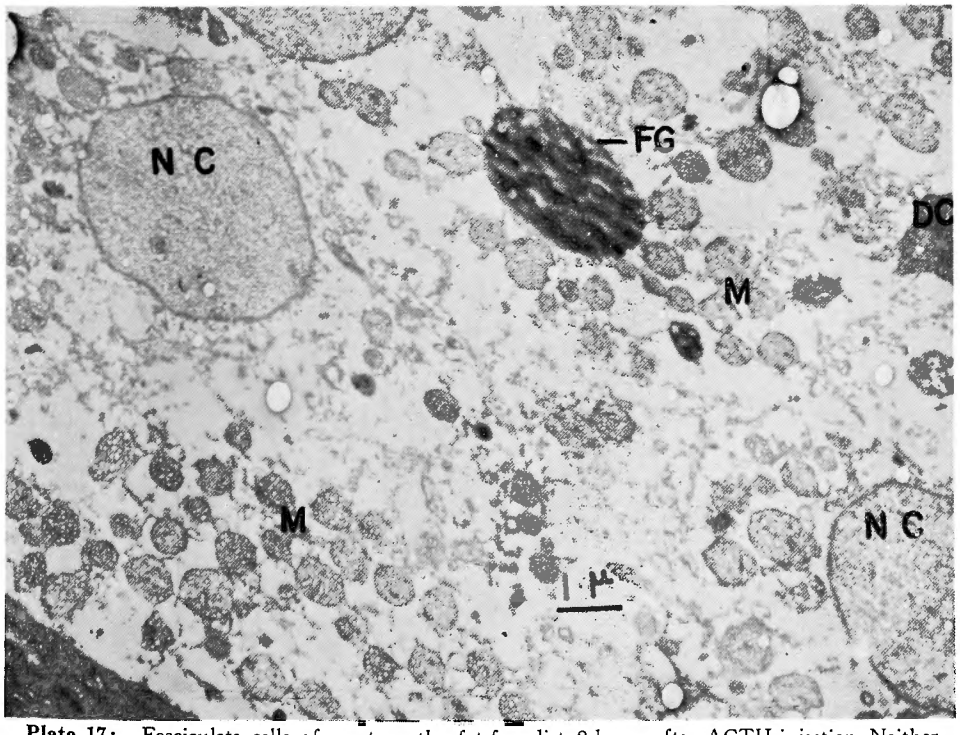

Plate 17: Fasciculata cells of a rat on the fat-free diet, 2 hours after ACTH injection. Neither increase in number of mitochondria nor fine fat granules are seen, only large ones. × 9000

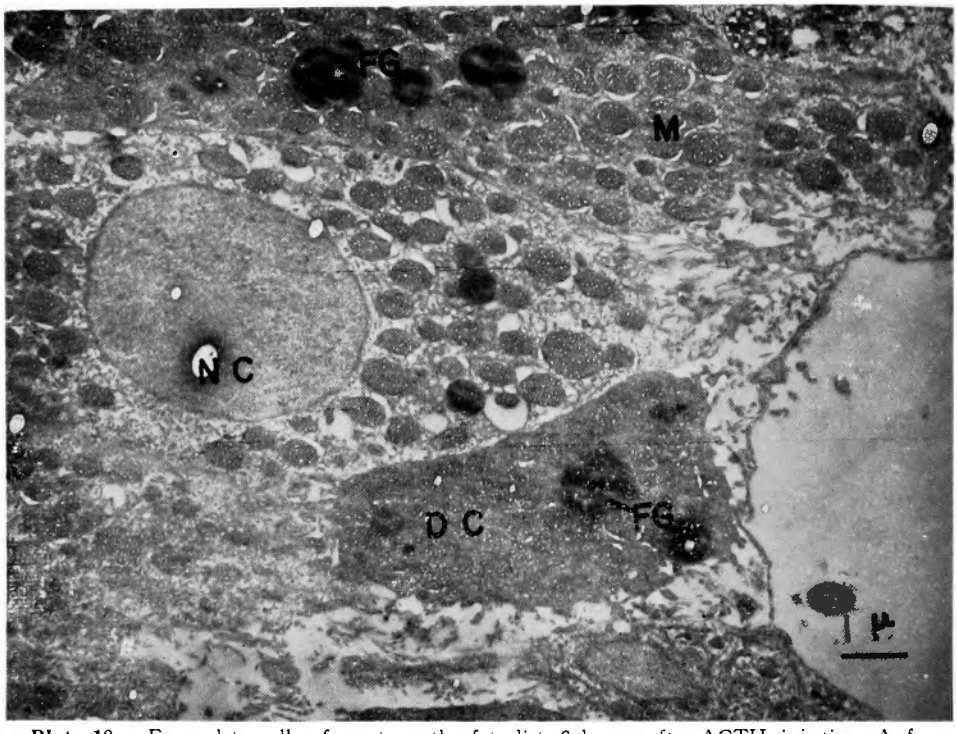

Plate 18 : Fasciculata cells of a rat on the fat diet, 6 hours after ACTH injection. A few fine fat granules 0.5 to 1.0 μ in size are seen and dark cells filled with many mitochondria are noted. × 9000

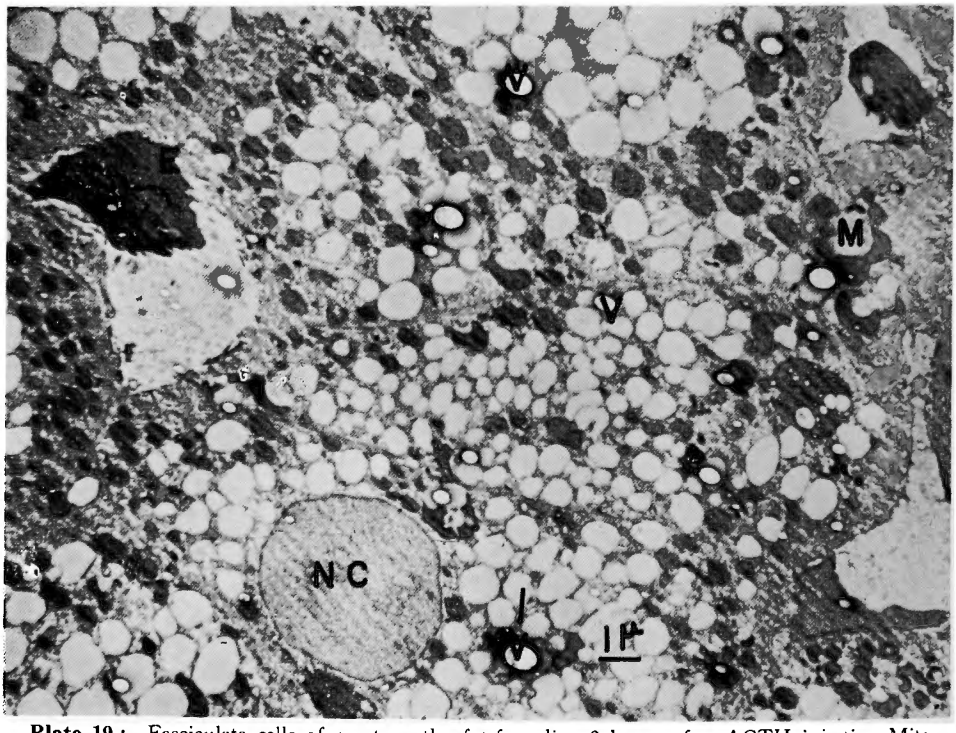

Plate 19 : Fasciculata cells of a rat on the fat-free diet, 6 hours after ACTH injection. Mitochondrial changes in both number and structure, and a large number of vacuoles are seen. × 6000

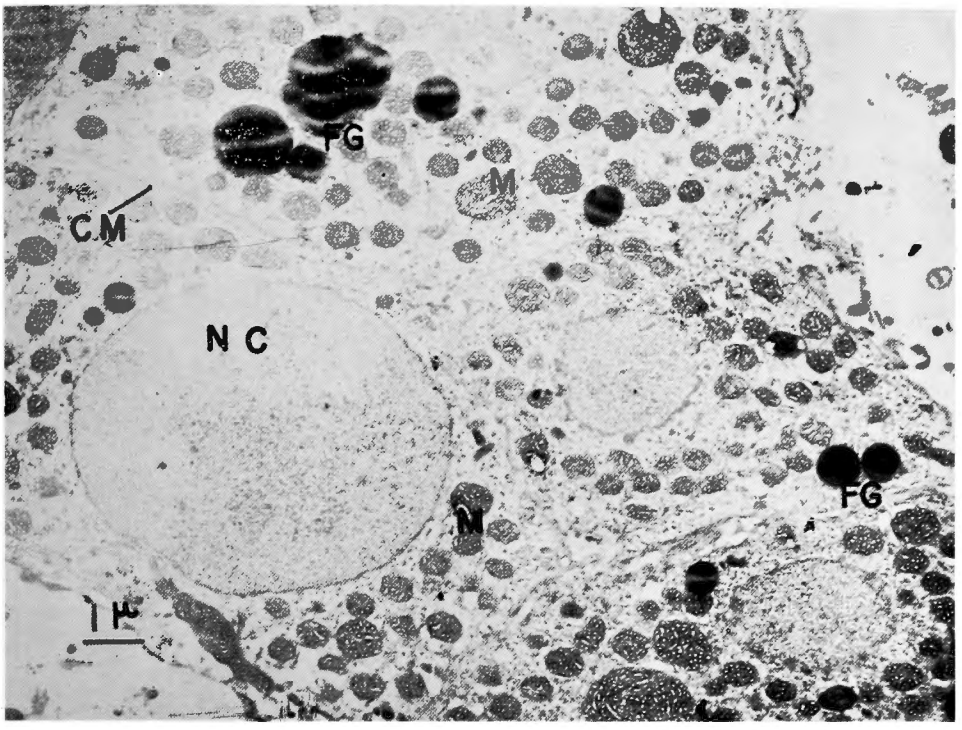

Plate 20 : Fasciculata cells of a rat on the fat diet, 12 hours after ACTH injection. Mitochondrial increase in number and fine fat granules persist. × 9000

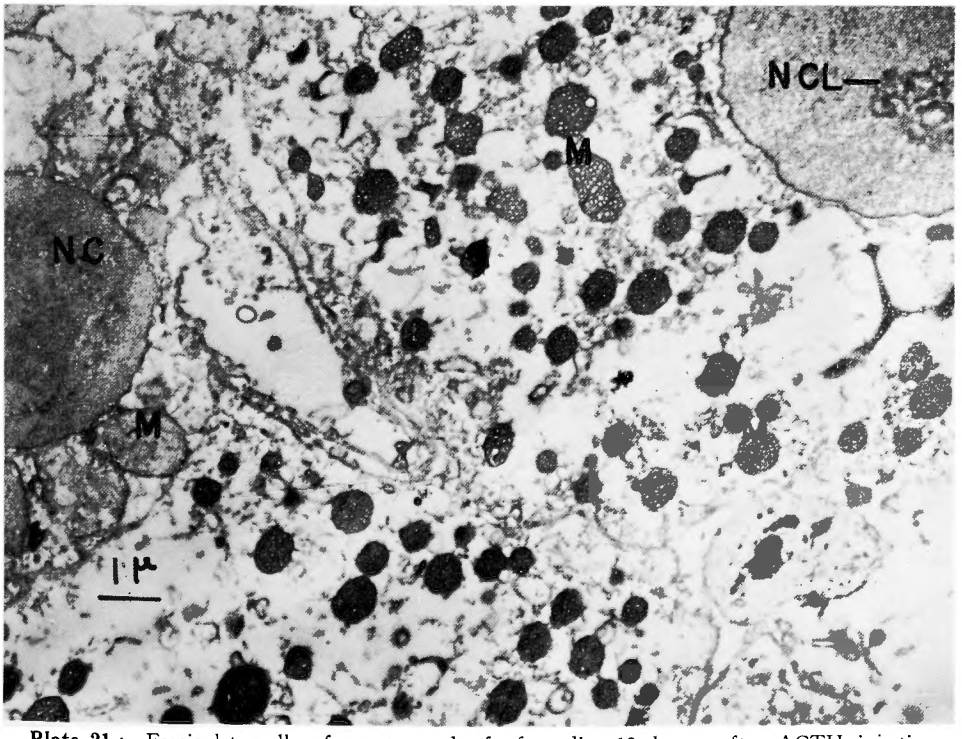

Plate 21 : Fasciculata cells of a rat on the fat-free diet, 12 hours after ACTH injection. Mitochondrial changes still persist. × 9000

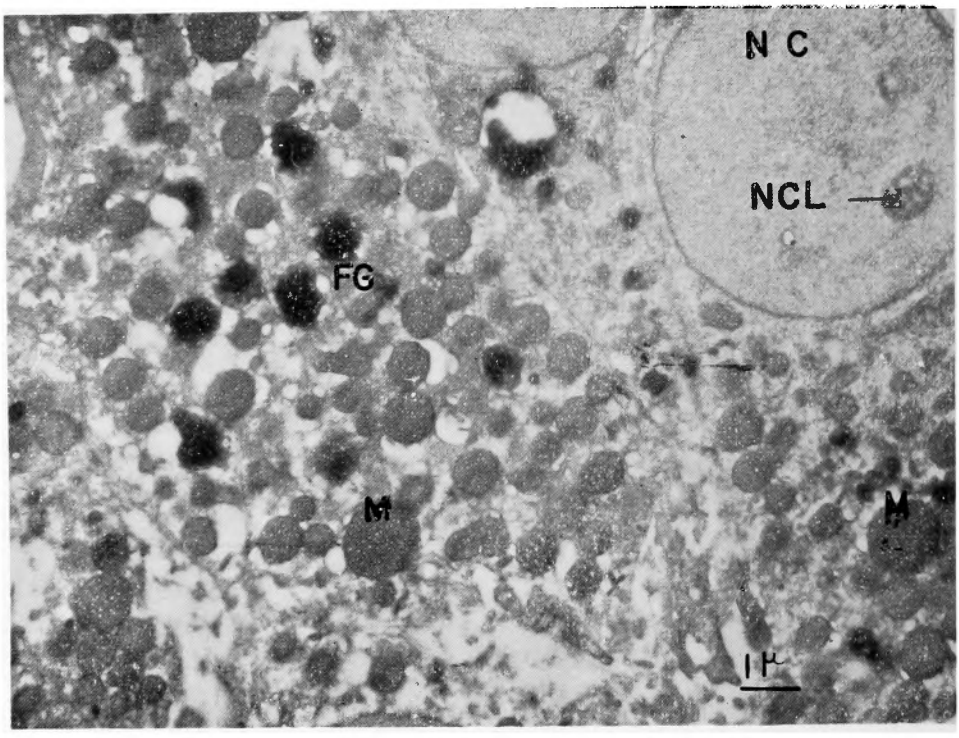

Plate 22 : Fasciculata cells of a rat on the fat diet, 24 hours after ACTH injection. × 9000

Plate 23 : Fasciculata cells of a rat on the fat-free diet, 24 hours after ACTH injection. Changes in the shape and structure of the mitochondria are still noted, and less dense osmiophilic granular substance is seen (arrow). × 9000

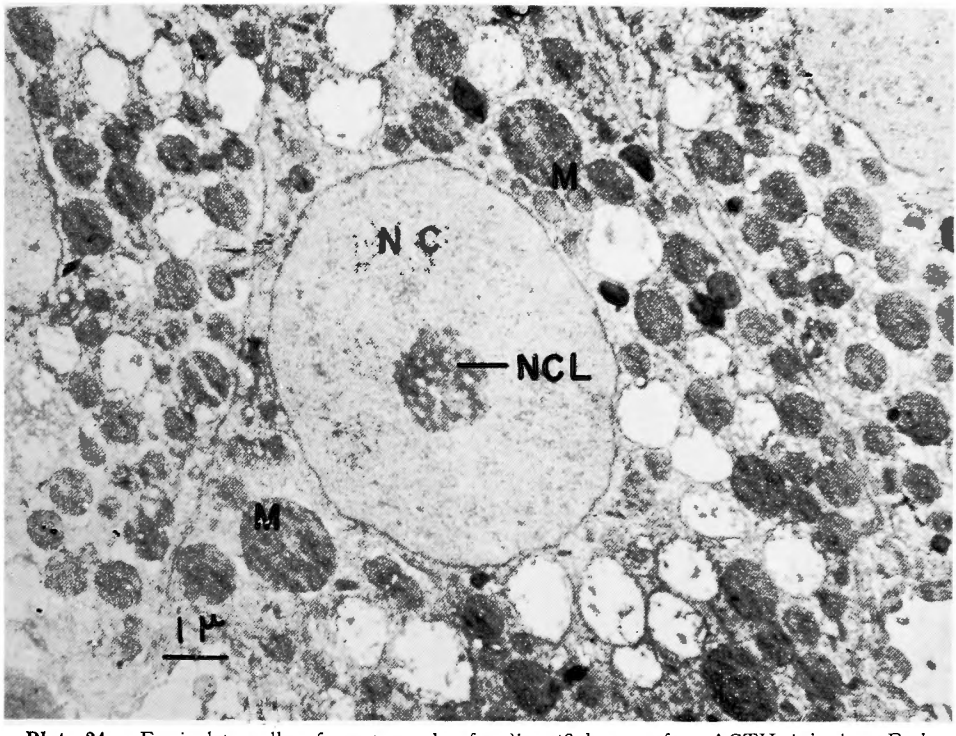

Plate 24 : Fasciculata cells of a rat on the fat diet, 48 hours after ACTH injection. Each cytoplasmic organella is returning to a resting state. × 9000

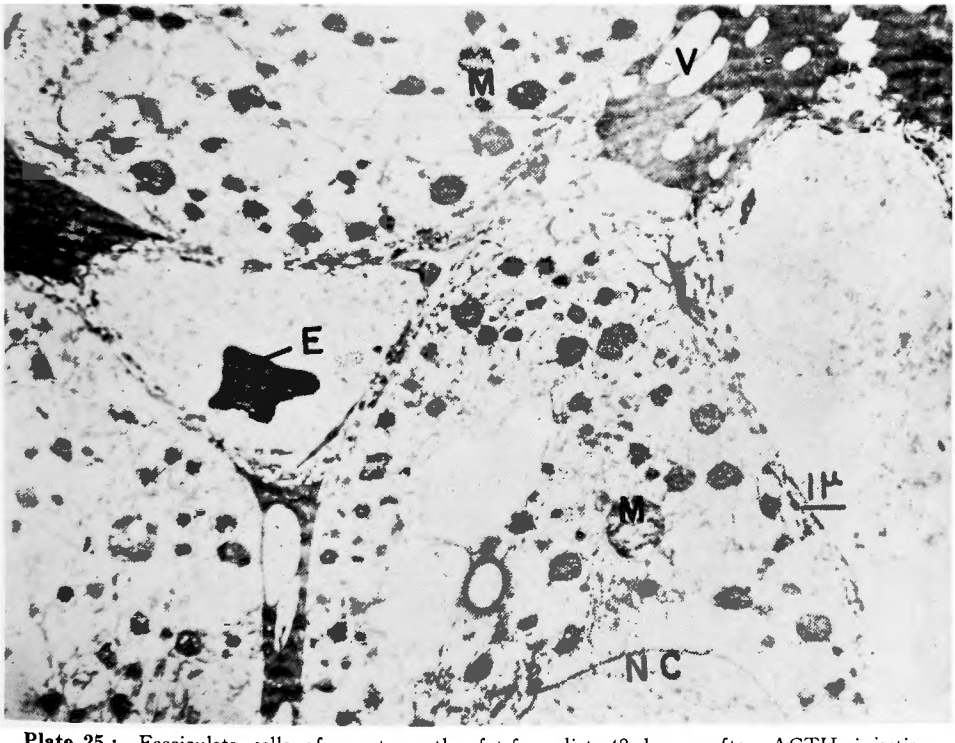

Plate 25 : Fasciculata cells of a rat on the fat-free diet, 48 hours after ACTH injection. Mitochondrial changes persist. × 6000

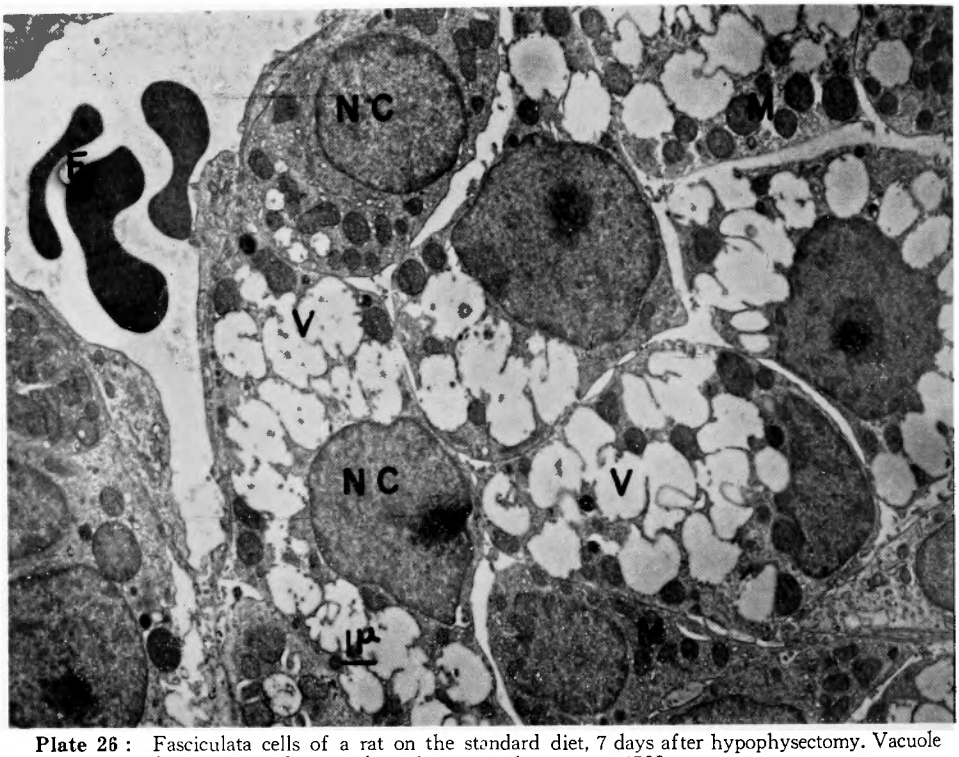

Plate 26 : Fasciculata cells of a rat on the standard diet, 7 days after hypophysectomy. Vacuole formation in the cytoplasm is progressing. × 4500

Plate 27 : Fasciculata cells of a rat on the standard diet, 14 days after hypophysectomy. Each cell is almost completely filled with many vacuoles, and mitochondria are rarely seen. × 4500

和文抄録

副腎皮質の電子顕微鏡学的研究

殊に不可欠脂酸の欠乏が副腎皮質像に及ぼす影響に就て

京都大学医学部外科学教室第2講座（指導：青柳安誠教授）

石　丸　久　生

従来から教室に於ては脂質の生体内代謝過程及びその栄養学的効果についての研究を行い，就中不可欠脂酸の生理学的意義の解明に努めて来た．

この間，松田が行なつた白鼠を一定期間絶食させた場合の肝糖原量及び副腎皮質の組織学的所見の推移，又長瀬が行なつた術後急性肺水腫の発生素因としての不可欠脂酸欠乏の意義についての研究成績は，何れも不可欠脂酸の欠乏は副腎皮質機能の低下を招来する大きな一因子となり得る事を暗示した．更に玉木が行なつた各種の飼料で飼育された白鼠の glucocorticoids 分泌量を指標として副腎皮質機能の状態を追及した研究成績は不可欠脂酸欠乏試獣は健常試獣に較べて極端にその副腎皮質機能が低下している事を明らかにした．

即ち以上の研究成績から，不可欠脂酸は副腎皮質ホルモンの生合成に重要な役割を果しており，従つて生体内不可欠脂酸の欠乏に際しては，当該個体の副腎皮質機能は著しく減弱し，健常時は兎も角としても，各種の stress 下に於ては当該個体の副腎皮質はその個体が要求するだけの皮質ホルモンの需要には応じ得なくなるものと考えられるのである．

本研究は上述の共同研究者が見出し得た生化学的並びに組織学的研究成績を，更に微細構造学的見地から再検討する意味で，各種の飼料で飼育した白鼠に対し各種の条件を負荷し，その際当該個体の副腎皮質，就中その束状層がいかなる所見を示すかを電子顕微鏡学的に追究したもので，その結果次の所見をえた．

(1) 不可欠脂酸欠乏試獣の副腎皮質束状層の細胞はその安静時にあつても，健常試獣のそれに較べると，糸粒体が大小不同となり，同時にその内部構造も乱れ勝ちである．

勿論，微細な脂質顆粒の数も少ない．

(2) 不可欠脂酸欠乏試獣に対し，各種の条件を負荷すると，当該試獣の副腎皮質束状層の細胞は早期から高度の細胞内オルガネラの疲憊性変化を示し且つその健常状態への復帰が著しく遅延する．

(3) 斯る細胞内オルガネラの示す疲憊性変化の主なものは，糸粒体の数の減少並びにその内部構造の変化，空胞形成，微細な脂質顆粒の消失，滑面小胞体の脱落等である．

(4) それに反し，副腎皮質機能の健常に保たれている脂質食群の試獣に於ては，たとえばその下垂体副腎皮質系機能を亢進せしめるなどの条件を負荷しても，糸粒体は増加し，滑面小胞体の発達が認められ，且つ微細な脂質顆粒が終始存在し，それが全く消失しつくす事は少なく，又粗大な脂質顆粒の出現を見る事もなく，速かに健常状態に復する．

(5) 従つて，副腎皮質機能を健常に保持するためには，不可欠脂酸が充分に投与されなければならない．要するに，個体の副腎皮質機能はそこに存在する不可欠脂酸量の如何によつて大きく左右される．

(6) 副腎皮質束状層の細胞内に存在する糸粒体の内部構造は，電子顕微鏡学的にみて steroidhormone の生合成に関与する臓器にみられるものと同様の構造を示して，その他の臓器にみられるものとは著しく趣きを異にしている．而して斯る束状層細胞内に存在する糸粒体は glucocorticoid の産生に大きな役割を演じていると考えられるものである．